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(57) Abstract

A method for producing feed for forming into pellets, and the feed therein referred to. The addition of the enzyme transglutaminase (EC 2.3.2.13) to a feed specially intended for carnivorous fish will catalyse a reaction between the amino acids glutamine and lysine which form part of the protein chains in the raw materials of the proteins of the feed, such that a covalent chemical bond is formed between them, which results in shape permanents in formed, dried, finished pellets, with the result that they do not lose their shape before their time of use.

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A METHOD FOR THE MODIFICATION OF PROTEIN STRUCTURE IN FINISH SHAPED FEED PELLETS, BALLS OR THE LIKE IN ORDER TO ACHIEVE SHAPE STABILITY, AND FEED MASS MADE IN ACCORDANCE WITH THE METHOD

The present invention relates to a method for the modification of protein structure in finish shaped feed pellets, balls or the like, among other reasons in order to contribute to strengthening the permanence of the pellet shape in granular feeds of this sort. The invention also relates to feedstuff manufactured according to this method for the formation of a shape-permanent feed in pellet form.

Many types of animals in breeding, e.g. salmon and trout, are carnivorous (meat-eating). Their natural food consists of insects (for salmon and trout in the freshwater phase), fish and crustaceans. For other carnivorous farmed animals, such as mink and foxes, the feed may also contain the mammalian flesh, for example fresh slaughterhouse waste. Insects and crustaceans have an exoskeleton which i.a. consists chitin. Chitin is a linear polysaccharide of N-acetyl-D-glucosamine linked by ß1→4 bonds. Other structural carbohydrates such as cellulose (ß[1→4] D-glycose) and alginate (D-mannuronic acid) are not found in these prey animals, nor do they con-

tain starch (α [1→4] D-glycose) as energy stores. Nevertheless, salmon, trout and other carnivorous fish and animals have enzymes (for example, amylase) that are capable of breaking down starch in the gut and making it digestible, but they may be less efficient in this respect than herbivorous (plant-eating) fish and animals.

In feeds intended for carnivorous fish it is usual to add between 8 and 25% carbohydrates, for example in the form of wheat or maize, as a binding agent. After pressing, but especially after extrusion, the starch in these carbohydrates will form a matrix or base mass which gives the pellets mechanical strength and shape permanance so that the shape of the pellets can be maintained after drying, further processing, storage and transport.

Carbohydrates are utilised in metabolism as a source of energy. The energy density of carbohydrates is lower than that of protein and fat (17.6; 23.9 and 39.8 MJ/kg respectively). The digestibility of carbohydrates is also lower in carnivorous fish, and declines as the proportion of complex carbohydrates in the feed (above 10%) increases. Experiments have shown that salmonids have no metabolic need for carbohydrates. If fat replaces carbohydrates as an energy source, a carbohydrate-free fish feed of this sort will contain more energy per unit weight, as long as the relative proportions of the other components are held constant.

In order to give feed pellets shape permanence and mechanical strength, as mentioned above, it is known to add a binding agent in the form of 8 - 25% carbohydrates, for example wheat and/or maize. After pressing or extrusion in the feedstuff

material there will be established a starch matrix of the desired strength.

Other techniques in connection with the forming of feed into pellets balls or the like, have also been described. According to U.S. Patent no. 4 935 250, for example, a gel or mass of alginate is also produced during the forming.

The patent literature includes descriptions of feeds and feed mixtures in which the mass consists of gelatine or caseinate. See, for example, British Patent no. 2 212 125.

There are also feeds in which the binding characteristics produced by the coagulation of native proteins are exploited; see NO 179 731.

Small feed particles can be produced with the aid of an agglomeration technique, which are based on the principle of aggregating extremely small particles into larger particles. This process does not utilise carbohydrates as a binding agent. The feed components are bound together through various forms of contact bonds between the solid particles in the feed. The different forms of contact bonds can vary from hydrogen bonds, adhesion and cohesion to capillary forces. New covalent bonds are not created in this process. This is an obvious disadvantage for the maintenance of the feed pellet's form and strength, because covalent bonds are stronger than other chemical bonds.

A serious disadvantage of agglomerated feeds is thus that the bonds are weak, and given the lack of a continuous matrix such pellets are friable and fragile. The agglomeration tech-

nique cannot be utilised to produce particles of feed in pellet form with a diameter larger than about 2.5 - 3.0 mm.

In order to be able to produce larger feed particles/fragments/pellets/balls, etc., we must abandon the agglomeration technique without addition of carbohydrate and again return to carbohydrate as a binding agent. In low concentrations, complex carbohydrates such as starch are digested by salmonids, for example, but if their concentration exceeds 10% the digestibility of the carbohydrate fraction decreases (Aksnes A., 1995. Growth, feed efficiency and slaughter quality of salmon, Salmo salar L., given feeds with different ratios of carbohydrate and protein. Aquaculture Nutrition, 1:241-248). The energy content of the carbohydrate fraction may be replaced by fat. This will result in greater freedom with respect to varying the relative proportions of fat, protein and micronutrients since the carbohydrates make up the remainder of the feed recipe. Such a feed will be richer in energy than an equivalent feed containing carbohydrates, and a reduction in the feed conversion ratio, defined as the quantity of feed consumed to produce one kilo of fish biomass, will be obtained.

In accordance with the present invention one has aimed at showing a method of modifying the protein structure of feeds whose nutrient composition closely resembles the natural choice of foods of carnivorous fish and animals. According to the invention, favourable binding is obtained in feeds - without carbohydrates - resulting in shape permanence in pellets and similar forms of feedstuff, and in such a way that the maximum particle size/pellet diameter can be increased in the case of agglomerated feed while maintaining the shape of

the feed. The invention also aims to increase the energy density of all types of feed, particularly fish feeds.

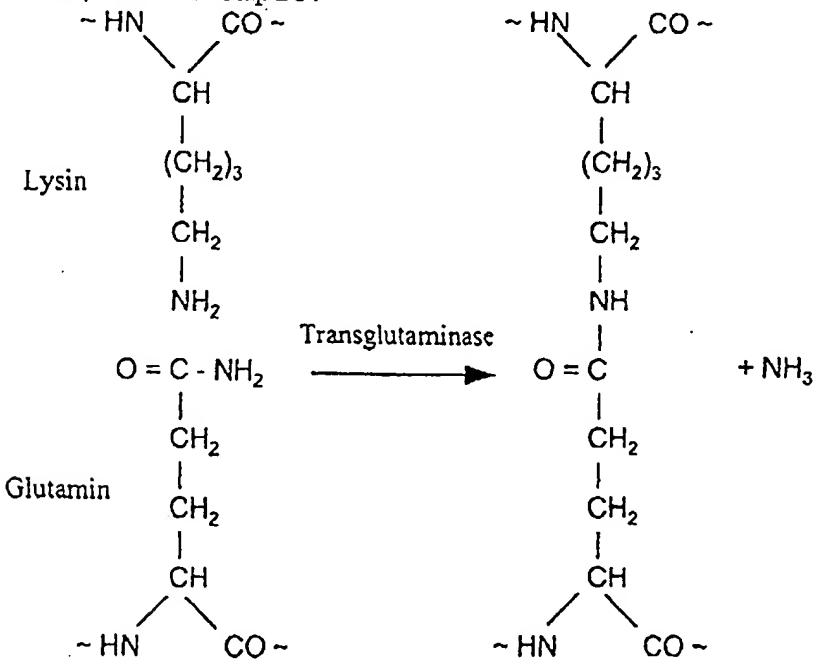
The said objective is reached by proceeding as described in patent claim no. 1. A feedstuff that has been treated as described in accordance with this method and which is intended for the forming of pellets or the like, is notable for the demonstration of properties that appears from the subsequent patent claim no. 7. Feed pellets made of such a feedstuff, whose protein structure has been modified by the method, also fall within the framework of the patent claims as these have been drawn up.

The invention thus consists mainly of a feedstuff, or alternatively of one or more of the components of which it consists, for forming pellets, alternatively of coating the surface of the formed pellets, in order to add an enzyme (preferably transglutaminase) that catalyses the formation of covalent bonds between the amino acids that make up the protein chains in protein raw materials containing proteins in native or denatured form. The raw materials of the protein in the feed mixture include the natural amino acids glutamine and lysine in the protein chains. The added enzyme will act as a catalyst and catalyze the formation of covalent (ε-(γ-Glu)Lys) bonds between the amino acids glutamine and lysine in the protein raw ingredients in the feed.

Through the adapting of reaction temperature and reaction time this enzymatic reaction will form a matrix or basic mass of protein raw materials which will exhibit adequate strength to give the feed pellets a constant and lasting shape.

In agglomerated feed this enzymatic reaction will lead to covalent transverse bonds (cross-bonds) between the proteins. This will give the agglomerate increased strength by introducing the strongest type of chemical bond in addition to the other three types of chemical bonds that give the feed product its firness and strength.

In pressed or extruded feeds, the formation of covalent (ε-(γ-Glu)Lys) bonds that lead to the formation of a protein matrix will be able to partially or wholly replace the addition of carbohydrates. This makes it possible to remove carbohydrates from the recipe for the feed, or to reduce the proportion of carbohydrates respectively. This is an important advantage from a number of points of view. Carbohydrates play virtually no part in the natural diet of carnivorous fish and animals, and the total energy density of feed pellets can be increased because both protein and fat are richer in energy than carbohydrates. According to the present invention, an animal feed in the form of pellets will require no other binding agents such as gelling agents in the form of gelatine, for example.



The above reaction formula, in which the enzyme transglutaminase acts as a catalyst, shows the creation of ε-(γ-Glutamyl) lysine bond between the amino acids glutamine and lysine.

Transglutaminase is defined as enzymes which are classified as protein-glutamine γ-glutamyltransferase (EC 2.3.2.13; International Union of Biochemistry and Molecular Biology, Nomenclature Committee). Transglutaminase may occur in a pure form or as a distinct premix with suitable filler and transglutaminase in adjusted concentration. Transglutaminase can be added to the other raw ingredients of the feed in the form of a powder, in solution or in suspension.

The term "protein raw materials" refers to raw ingredients that contain protein in either native or denatured form. Examples include fish meal, stickwater, stickwater concentrate, blood meal, feather meal, bone and horn meal, wheat gluten, maize gluten, soya meal and rapeseed meal. These are only illustrative examples, and do not exclude the use of other raw ingredients in feeds as partly substitute for or additions to one or more of the said raw ingredients.

The term "feed pellets" refers to particles or fragments, preferably round, which are formed by means of a special process, such that they are of a size and shape that makes them suitable as feed, particularly for carnivorous fish species such as farmed salmon, cod, halibut, sea perch and sea bream.

The activity of the enzyme (transglutaminase) declines at temperatures higher than 50° C, and it is deactivated at temperatures beyond 65° C. The formation of a protein matrix as described here can thus take place under process heat condi-

tions in which the process temperature varies betwen 0 and 60° C. The shortest process time is obtained at around 50° C, since the process time is lengthened at temperatures above and below this temperature.

Transglutaminase is added to the other ingredients of the feed before these are formed into pellets by a suitable method, since the enzyme can be added as a solution or as a suspension in a suitable liquid, for example water, or blended as a dry ingredient before liquid is added to the mixture. In addition to transglutaminase, an aqueous solution may contain pure water, stickwater or stickwater concentrate, or another protein-rich liquid, for example a non-limiting gelatine solution.

Transglutaminase can also be added to the surface of pre-formed pellets by a suitable method, in that the enzyme can be added dissolved in steam, or as a solution, suspension or wash in a suitable liquid such as water.

The fat content of the feed may, according to a non-limiting preferred example, consist of fish-oil, which can be added to the feed either before or after it is formed into pellets, or after these have been dried. After the feed has been formed into pellets, these are maintained at a temperature of between 0 and 60° C, so that the transglutaminase enzyme has time to catalyse the ε-(γ-Glutamyl) lysine bonds that are desired.

The reaction time is adapted to the reaction temperature.

Finally, the formed pellets are dried to the desired water content/degree of dryness in a suitable dehydration unit, such as a drying cabinet.

The transglutaminase product used in the following examples (from the manufacturer Ajinomoto in Japan) consists of 60% sodium caseinate, 39.5% maltodextrin and 0.5% transglutaminase. A meat grinder with a die size of around 6.5 mm can be used to form the feed into pellets. The apparatus may also comprise an incubator cabinet and a drying cabinet (working temperature about 80 - 90° C). In a wear test, a rotation rate of 500 rpm was employed without the use of metal balls.

Example 1

500 g fish meal (Norwegian LT meal) and 500 ml water were mixed to formation of a dough which was then formed into pellets with the aid of a meat grinder. The formed pellets were dried for about 35 minutes. 300 g pellets were used for the wear test.

25 g transglutaminase product containing 125 mg transglutaminase and 15 g sodium caseinate was stirred into 500 ml water at about 40° C. The solution was added to 500 g fish meal (Norwegian LT meal), mixed to a dough and formed into pellets with the aid of a meat grinder. These pellets were incubated in the said incubator cabinet for about 60 minutes at about 40° C, and thereafter dried for about 50 minutes. 300 g pellets were used for the wear test.

Result

Composition of fish meal	protein fat ash water	72.5% 8.7% 11.8% 8.5%
Water content after drying	meal + water meal + sodium caseinate + water + transglutaminase	3.3% 3.6%
Wear test, remaining pellets	meal + water meal + sodium caseinate + water + transglutaminase	1.2% 86.3%

The example shows that blending around 125 ppm transglutaminase into a mixture of fish meal, sodium caseinate and water produced pellets with considerably greater firmness and strength than pellets formed from a feed mass (fish meal + water) without this enzyme additive.

The enzyme transglutaminase in powder form can be blended with one or more of the other dry ingredients of the feed before water is added in the form of liquid, for example pure water, stickwater, stickwater concentrate, other protein-enriched liquid or water vapour.

Drying may take place immediately after forming into pellets, as long as care is taken to ensure that the temperature does not rise above 60° C, thus giving the enzyme sufficient time to act to create a protein matrix before the water activity

becomes so low that the enzyme will no longer act as a catalyst.

Transglutaminase produced by temperature-tolerant bacteria will be able to act at temperatures higher than 60° C.

Production conditions can thus be above 60° C if thermostable transglutaminase is employed.

Example 2

In this case another source of protein (soya) was used, whose real protein level is about 20% lower than that of fish meal. The feedstuff was prepared largely as indicated in Example 1.

500 g soya meal (Hamlet) and 700 ml water were mixed to the formation of a dough, which was formed into pellets and dried for about 40 minutes. As in example 1, a wear test was carried out. After the test had been completed, the remaining whole pellets were subjected to a new wear test, this time with four metal balls and at 150 revolutions.

25 g transglutaminase product containing 125 mg transglutaminase and 15 g sodium caseinate was stirred into 700 ml water at about 40° C. The solution was added to 500 g soya meal flour, blended and formed as indicated in example 1, incubated at 40° C for 60 minutes and dried for about 60 minutes.

Result

Composition of soya meal	protein fat ash water	56.5% 1.0% 7.1% 7.9%
Water content after drying	meal + water meal + sodium caseinate + water + transglutaminase	10.0% 7.2%
Wear test without balls, remaining pellets	meal + water meal + sodium caseinate + water + transglutaminase	85.6% 95.8%
Wear test without balls, remaining pellets	meal + water meal + sodium caseinate + water + transglutaminase	35.5% 72.0%

The invention also comprises other enzymes that catalyse the formation of covalent bonds between amino acids that form part of the protein chains in the raw materials of the protein in the feed.

Such an enzyme can, for example, catalyse an equivalent reaction between asparagine and lysine (ε-(ß-aspartyl) lysine bond) (transasparaginase).

Other examples are the enzyme protein disulphide isomerase (EC 5.3.4.1), which catalyses the rearrangement of cystine and the enzyme lipoxygenase.

C l a i m s

1. A method for modifying the protein structure of preformed feed pellets, spherical pellets and similar feed particles or fragments in order to achieve shape permanence, strength and firmness in the said feed pellets and similar products, where the said properties are retained after possible incubation, drying, possible further processing, storage and transport,

c h a r a c t e r i z e d i n that an enzyme is admixed that catalyses the formation of covalent bonds between animo acids that form part of the protein chains in the raw material of a protein containing proteins in native or denatured form, and which may for example be fish meal, stickwater, stickwater concentrate, blood meal, feather meal or bone meal, wheat gluten, maize gluten, soya meal, rapeseed meal, casein, sodium caseinate, gelatine or collagen to the premixed feed mass or alternatively to one or more of its ingredients before blending, whereafter the feed mass to which the catalyst in the shape of the said enzyme is formed into feed pellets or similar products, alternatively that the said enzyme is added to the surface of preformed feed pellets which are incubated at a temperature and for a period of time appropriate to the kinetic properties of the enzyme so that covalent bonds are created between the said amino acids, and that the said feed pellets or the like are further treated, among other things being dried by a known method, and as preformed feed pellets, feed balls and similar feed particles or feed fragments display the desired quality of shape permanence.

2. A metod as claimed in claim 1, c h a r a c t e r i z e d i n that transglutaminase or alternatively transasparaginase, protein disulphide isomerase or lipoxygenase are utilised as catalysing enzymes, and the amino acids glutamine (alternatively asparagine) and lysine or alternatively cystine form part of the chains of raw materials in the protein of the feed.

3. A method as claimed in claim 2, c h a r a c t e r i z e d i n that the enzyme transglutaminase is admixed with the feed in the amount of at least 10 ppm of the (dry) weight of the feed, and after forming into pellets and similar products a reaction temperature is obtained within the range of 0 - 60° C, preferably 10 - 50° C (apart from thermostable transglutaminase, which is capable of acting as a catalyst at temperatures >60° C), whereupon the feed pellets are incubated and dried (for example for 60 and 50 minutes respectively, depending on the temperature), so that the catalytic properties of the enzyme cease to be operative when the said feed pellets are fully dried.

4. A method as claimed in claim 3, c h a r a c t e r i z e d i n that the enzyme transglutaminase is admixed in the form of a powder to the dry feed or alternatively to one or more of its ingredients before blending, and is mixed into the said feed before water is added to the mixture in the form of a liquid, such as pure water, stickwater, stickwater concentrate, other protein enriched liquid and/or water vapour.

5. A method as claimed in claim 2, c h a r a c t e r i z e d i n that to a blending liq-

uid for the feed (which is of approximately the same weight as the last-mentioned), is added about 20 ppm or more transglutaminase, relative to the weight of the blending liquid, the said transglutaminase being stirred into the blending liquid to create an aqueous solution, or suspension, which is blended with a dry mass of feed of approximately the same weight as the blending liquid.

6. A method as claimed in claim 1, c h a r a c t e r i z e d i n that the enzyme transglutaminase dissolved in water vapour, as a solution, a suspension or a wash in a suitable liquid, for example water, is added to the surface of preformed feed pellets, and after forming into pellets and similar products a reaction temperature is established within the range of 0 - 60° C, preferably 10 - 50° C (apart from thermostable transglutaminase, which is capable of acting as a catalyst at temperatures >60° C), whereafter the feed pellets are incubated and dried (for example for 60 and 50 minutes respectively, depending on the temperature), so that the catalytic properties of the enzyme cease to be operative when the said feed pellets are fully dried.

7. A feed mass for the formation of feed particles (pellets) for farmed carnivorous animals, prepared and treated in accordance with the method of any one of the above claims, c h a r a c t e r i z e d i n that an enzyme is added to the feed, in particular transglutaminase, for the catalysis of a reaction between amino acids, particularly glutamine and lysine, which form part of the protein chains in the raw materials of the proteins of the feed, which reaction results in the formation of covalent

cross-bonds between proteins, and which feed contains among other ingredients proteins and micronutrients.

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A23K 1/10, A23K 1/18, A23K 1/165, A23L 1/31, A23J 3/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A23K, A23L, A23J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CAPLUS, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	Patent Abstracts of Japan, abstract of JP 62-61692 A (AJINOMOTO CO INC), 20 Sept 1994 (20.09.94) --	1-7
X	Patent Abstracts of Japan, abstract of JP 58-28234 A (YUKIJIRUSHI NIYUUGIYOU KK), 19 February 1983 (19.02.83) --	1-7

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

Information on patent family members

01/07/99

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